Report as of FY2009 for 2009PA93B: "An Emerging Technology for Emerging Contaminants: Biocatalysis of Endocrine Disrupting Chemicals in Wastewater for Beneficial Reuse"

Publications

- Articles in Refereed Scientific Journals:
  - Henry, E., Caporuscio, A. F, and Brennan, R. A. (2010) "Optimum conditions for the biodegradation of endocrine-disrupting compounds in wastewater by Phanerochaete chrysosporium." Manuscript in preparation for Science of the Total Environment.

Report Follows

FY09 PROJECT REPORT (FINAL REPORT)
Pennsylvania Water Resources Research Center

An Emerging Technology for Emerging Contaminants: Biocatalysis of Endocrine Disrupting Chemicals in Wastewater for Beneficial Reuse

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PRINCIPAL FINDINGS AND SIGNIFICANCE

As beneficial water reuse becomes a common practice throughout the world, concern over the effects of residual contaminants on aquatic ecosystems and human health is escalating. Found in everyday commercial items like plasticizers, pharmaceuticals, pesticides, and flame retardants, endocrine disrupting chemicals (EDCs) have been shown to disrupt hormone function in exposed organisms, causing adverse physiological problems. Typically, these contaminants are not completely removed during conventional wastewater treatment, and are discharged into receiving waters, where they can potentially harm ecosystems and reenter potable water supplies. Although some physical- and chemical-treatment methods exist for treating EDCs in wastewater, they are expensive and unattainable for the majority of the world. An inexpensive, sustainable treatment method is sorely needed for removing residual contaminants from wastewater effluent.

The overall goal of this research is to test the effectiveness of enzymatic biocatalysis for the reduction of EDCs in wastewater effluent using fungal mycelia. The white-rot fungus *Phanerochaete chrysosporium* (ATCC 24725) was chosen for this study based on its previously documented ability to degrade multiple environmental contaminants at high rates. It was theorized that with the application of *P. chrysosporium* mycelia to secondary wastewater treatment plant effluent, the enzymes lignin peroxidase (LiP) and manganese peroxidase (MnP) would be produced which would catalyze the removal of EDCs from solution. To begin to test this hypothesis, a series of continuously aerated batch tests were conducted in a standard factorial design to rapidly assess the ability of immobilized fungal mycelia to achieve enzyme production under different conditions. The variables evaluated included 1) indigenous microbial activity, 2) pH, and 3) organic carbon. Non-sterile and sterile treatments were compared to determine how indigenous wastewater microorganisms would affect fungal growth and enzyme activity. Comparisons were also made between the natural wastewater pH (~7.4) and the "optimum" pH for *P. chrysosporium* (~4.3). Finally, the addition of glucose was evaluated to determine if supplemental carbon is needed to support the growth and activity of *P. chrysosporium* in wastewater.

The principal findings and significance of this work include the following:

1. Growth and attachment *P. chrysosporium* occurred in non-sterile, aerated wastewater reactors under optimal pH conditions. Within these reactors, extracellular enzyme activity (LiP and MnP) was induced. In order to sustain and increase enzyme

activity, multiple supplementations and/or various concentrations of veratryl alcohol should be evaluated.

2. Adjusting the pH to the optimum range (4.0 – 4.5) is necessary to: 1) obtain and maintain attached growth; and 2) produce enzyme activity. In long-term batch experiments, reactors should be buffered to maintain a pH within the optimum range.

3. Glucose amendments only appeared to positively affect the attached growth of the sterile reactors that had been pH adjusted to 4.3. In future experiments, it should be possible to grow *P. chrysosporium* and produce enzyme activity without the addition of glucose.

Experiments currently in progress are evaluating the effects of wastewater treatment stage, temperature, nutrient supplementation, and traditional vs. sustainable support structures for mycelia attachment. After optimizing treatment conditions in batch mode, bioreactor studies will be conducted to quantify EDC removal rates, and confirm suitable hydraulic residence times when immobilized mycelia are used for EDC treatment. If successful, this work will be the first to utilize fungi-driven biocatalysis for the remediation of EDCs in wastewater, which could provide a more sustainable, cost-efficient alternative over traditional removal processes.

STUDENTS & POSTDOCS SUPPORTED

1. **Erin Henry**, "Optimization of conditions for the degradation of endocrine-disrupting compounds by white-rot fungi in large-scale wastewater applications", Department of Civil & Environmental Engineering, Penn State, M.S. expected December 2010.

2. **Abby Caporuscio**, "Enzymatic biocatalysis of endocrine disrupting chemicals in wastewater using *Phanerochaete chrysosporium*", Department of Civil & Environmental Engineering, Penn State, M.S. expected December 2010.

3. **Michael Shreve**, "Sustainable growth media for the fungal remediation of endocrine disruptors in municipal wastewater", B.S. Environmental Systems Engineering with Honors in Environmental Engineering, expected December 2010.

4. **Dr. Neil Brown**, "Pharmaceutical removal from wastewater using ecological processes", Department of Civil & Environmental Engineering, Penn State, research associate, 2008 – present.

PUBLICATIONS

1. Henry, E., Caporuscio, A. F, and Brennan, R. A. (2010) "Optimum conditions for the biodegradation of endocrine-disrupting compounds in wastewater by *Phanerochaete chrysosporium*." Manuscript in preparation for *Science of the Total Environment.*

PRESENTATIONS AND OTHER INFORMATION TRANSFER ACTIVITIES

1. Brennan, R. A. (2010) "Using Living Machines to Remove Endocrine Disruptors from Wastewater." *Invited seminar*, The Pennsylvania Water Symposium: Groundwater and Surface Water: A Single Resource, University Park, PA, May 6.

2. Henry, E., and Brennan, R. A. (2010) "Determination of optimum conditions for removal of endocrine-disrupting compounds by white-rot fungi in large-scale applications." *Poster presentation*, The Pennsylvania Water Symposium: Groundwater and Surface Water: A Single Resource, University Park, PA, May 6.

3. Caporuscio, A. F., and Brennan, R. A. (2010) "Magnificent mushrooms: enzymatic biocatalysis of endocrine disrupting compounds in wastewater using fungal mycelia." *Poster presentation*, The Pennsylvania Water Symposium: Groundwater and Surface Water: A Single Resource, University Park, PA, May 6.

4. Brennan, R. A. (2010) "You're Going to Drink *WHAT*???: Using Living Machines to Clean Contaminated Water." *Invited seminar*, Penn State Lectures on the Frontiers of Science, sponsored by the Penn State Eberly College of Science and the Penn State Institutes of Energy and the Environment, The Pennsylvania State University, University Park, PA, Feb. 6.

AWARDS

1. Henry, E., and Brennan, R. A. (2010) "Optimum conditions for biodegradation of endocrine-disrupting compounds by white-rot fungi in large-scale wastewater applications." *Podium presentation (**Student Research Award Recipient**)*, Pennsylvania Water Environment Association 82nd Annual Technical Conference & Exhibition (PennTec 2010), State College, PA, June 14.

2. Caporuscio, A. F., Brown, N., and Brennan, R. A. (2010) "From silent spring to the forest floor: enzymatic biocatalysis of endocrine disrupting compounds in wastewater using fungal mycelia." *Poster presentation (**Student Research Award Recipient**)*, Pennsylvania Water Environment Association 82nd Annual Technical Conference & Exhibition (PennTec 2010), State College, PA, June 14.

ADDITIONAL FUNDING ACQUIRED USING USGS GRANT AS SEED MONEY

Source: Penn State Sustainability Seed Grant Program (PSIEE)
Start date: May 15, 2010
End date: June 30, 2012
Title: Life Cycle Assessment of Sustainable Wastewater Treatment Strategies: Toward the Development of an Enhanced Water-Energy Infrastructure

PHOTOS OF PROJECT

Figure 1. Support structures under consideration for mycelia attachment. From left to right: Jaeger Tri-packs ®, Fuzzy Filters™, rye grain, and wood chips.

Figure 2. Aerated batch microcosms used for testing fungal growth and enzyme production in wastewater.

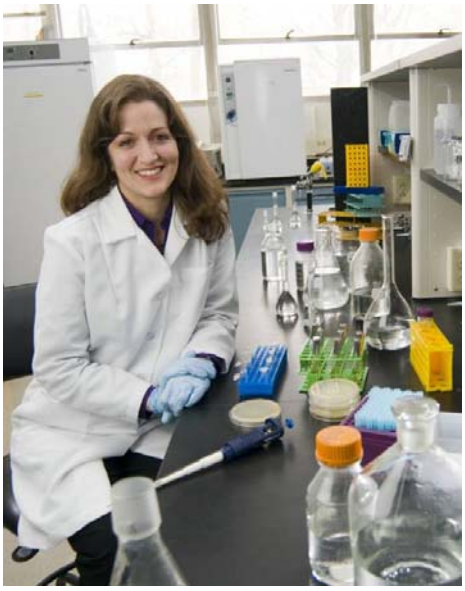

Figure 3. The principal investigator of this project, Dr. Rachel Brennan, in the lab.